Section 2: Executive Summary:

BACKGROUND: Diffuse intrinsic pontine glioma (DIPG) is the leading cause of brain cancer associated deaths in children despite constituting only 10-15% of childhood brain tumours. Approximately 300 children per year with a median age of 6-7 years will be diagnosed with DIPG in the US [about 20 per year in Australia]. Currently, radiotherapy is the only treatment offered as no effective chemotherapeutic is available and surgery is not an option due to tumor location. As a result, the median survival time is less than 12 months. This statistic unfortunately has not changed in over 35 years of investigation and highlights an urgent need for new and novel methods of targeting DIPG.

SCIENTIFIC MERIT AND FEASIBILBITY: Our research program identifies targeted therapeutics, both small molecule inhibitors and monoclonal antibodies, directed to cell-surface receptor tyrosine kinases (RTKs) that have potential efficacy in brain cancer. We are now beginning to apply this expertise to DIPG. DIPG is a highly heterogeneous tumor with a variety RTKs responsible for tumor initiation (PDGFRα), growth (EGFR), vascularisation (VEGFRs), dissemination (c-Met) and stem/progenitor cell maintenance (c-Kit). Although a number of clinical trials are under way using small molecule inhibitors and humanised monoclonal antibodies targeting EGFR with nimotuzumab [1], VEGFR and EGFR with Vandetanib [2] and PDGFR with Dasatinib [3], most have had limited success and appear to target one or two oncogenic proteins. In the first part of our proposal we will use a multi-targeting agent (AMG706 targeting PDGFRs, VEGFRs, c-Kit) and the single targeting agents AMG102 (targeting HGF, the c-Met ligand) and panitumumab, which targets wild type EGFR (EGFRwt) and the truncation mutant EGFRvIII), alone and in combination, to inhibit multiple cellular subpopulations and multiple growth promoting RTKs expressed in DIPG. In addition, we will investigate the novel anti-tumor activity of human amniotic stem cells (hASC) in DIPG, as these cells have demonstrated anti-RTK activity and apoptosis inducing activity in glioblastoma multiforme (GBM) *in vitro* and *in vivo*. Our extensive expertise in brain cancer research and access to novel therapeutic agents makes this proposal scientifically significant and highly feasible.

HYPOTHESES:

1: Targeting multiple activated RTKs in DIPG will be an effective therapeutic strategy;
2: hASC will have anti proliferative and anti-tumorigenic effects against DIPG sphere lines;
3: The combination of both these strategies will have enhanced anti-tumor activity in DIPG.

SPECIFIC AIMS

We will determine:

1: the anti-tumor activity of AMG706 in combinations with AMG102 and panitumumab against a panel of patient derived DIPG sphere cell lines *in vitro* and *in vivo* by conducting tumorigenicity-related functional assays (e.g. proliferation, cell cycle, apoptosis assays), and assessing RTK expression and activation, and orthotopic xenograft growth;
2: the anti-tumor activity and mechanisms of action of hASC against DIPG sphere cell lines *in vitro* and *in vivo* as described in Aim 1;
3: the anti-tumor activity of the therapeutic strategies in Aims 1 and 2 in *in vivo* subcutaneous and orthotopic xenograft models of DIPG.

PROJECT GOALS: To identify novel anti-tumor therapeutic strategies for DIPG

DESIGN AND METHODS: Our study will utilize primary DIPG sphere cell lines isolated from autopsied material to evaluate new therapies both *in vitro* and in orthotopic xenograft models of DIPG.

CLINICAL SIGNIFICANCE: This study will evaluate several targeted therapeutics (some that are in clinical trials and one that is approved by the Food and Drug Administration) that recent discoveries suggest should have anti-tumor activity in DIPG. As these therapeutics are already in the clinic, and because we will evaluate them in clinically relevant DIPG xenograft models, there will be the opportunity to rapidly translate them into the clinic. In this proposal, the therapeutic value of hASC in DIPG will also be assessed. These cells are already being used clinically in our institute; therefore, if our data indicate that they have therapeutic potential, we have the ability to conduct a trial in patients with DIPG. In the long term, we hope to design new therapeutic strategies that extend the lives of patients with DIPG.